SYNOPSIS OF PROPOSED RESEARCH
FOR
DOCTORAL DEGREE IN BIOINFORMATICS

Title of the research work

"Study on small molecule inhibitors of Activator Protein-1 involved in inflammatory diseases: *Insilico* screening and validation"

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SYNOPSIS

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Introduction

AP-1 was first identified as a TPA-activated transcription factor which bound to a cis-regulatory element of the human metallothionein IIa (hMTIIa) promoter and SV40. The AP-1 binding site was distinguished as the 12-O-Tetradecanoylphorbol-13-acetate (TPA) response element (TRE) with the consensus sequence 5'-TGA G/C TCA-3'. The AP-1 subunit Jun was keyed out as a novel oncoprotein of avian sarcoma virus and Fos-associated p39 protein was keyed out as the transcript of the cellular Jun gene. Since its discovery, AP-1 has been found to be associated with numerous physiological and regulatory processes and new relationships are still under investigation.

Activator protein 1 (aka activating protein 1, AP-1) is a censorious transcription factor which involves in a wide range of cellular processes including apoptosis, cell migration, proliferation, differentiation, transformation and survival(3). The AP-1 family of transcription factors consists of basic-leucine zipper protein families that are composed of multiple members of Fos (c-Foc, FosB, Fra-1 and Fra-2) and Jun (c-Jun, JunB and JunD)(14,15). The members of Fos are heterodimerize only with Jun members whereas the members of Jun are either homodimerize with Jun itself or heterodimerize with different Fos members(15). Thus, to form AP 1 complexes Jun is the predominant partner such as Jun:Jun or Jun:Fos dimmers. Via their leucine-zipper domains, these basic-region leucine-zipper (B-ZIP) transcription factors are dimerize, which in turn bring together their basic domains to bind DNA in a sequence manner. At the transcriptional level, the AP-1 complex converges at multiple points and making it a critical connecting node for many signal transduction pathways.

AP-1 has appeared as an actively pursued drug discovery target. Collective evidences suggests that AP-1 plays an important role in several disorders including fibrosis, organ injury, cancer as well as inflammatory disorders such as psoriasis, asthma, transplant rejection and rheumatoid arthritis (RA). In considering the great therapeutic potential of this target and the immense academic and industrial efforts dedicated to it, only one selective AP-1 inhibitor has been advanced into human clinical trials i.e 3-{5-[4-(Cyclopentyloxy)-2-hydroxybenzoyl]-2-[(3-hydroxy-1,2-benzisoxazol-6-yl)methoxy]phenyl} propionicacid (T-5224), is a non-peptidic small molecule AP-1 inhibitor that specifically inhibits the binding of AP-1 to the AP-1 binding site of the promoter region and was originally developed as an anti-

inflammatory drug for the treatment of rheumatoid arthritis (RA), MMPs and suppressing inflammatory cytokines without any side effects [1,2]. T-5224 is a novel AP-1 inhibitor co-developed by Toyama Chemical and Kitasato University, which has proven to prevent pannus formation, joint destruction and osteoclastogenesis in collagen-induced arthritis (CIA) in rats. T-5224 shows good outcome as a new drug for the treatment of arthritis and is currently in phase II human clinical trials in japan[3]. Addition to that, T-5224 is under investigation for other inflammatory diseases in which AP-1 is involved [3].

Inflammation is a very important part a body's immune system response to harmful stimuli such as pathogens, irritants or damaged cells, and it is also a protective response involving blood vessels, immune cells, and molecular mediators. Inflammation attracts the immune cells and induces the local production of several chemokines (e.g. IL-8) and cytokines (e.g. TNFα), expression and over production of pro-inflammatory cytokines stimulate the systematic inflammation, resulting to the development of many inflammatory diseases, such as rheumatoid arthritis, asthma and bowel diseases. For the treatment of chronic inflammatory diseases glucocorticoids (gcs) have been extensively used from the last half century. Glucocorticoids are the most widely prescribed and most effective anti-inflammatory medications that are presently available. The anti-inflammatory effects of glucocorticoids are due to their ability to repress the inflammatory gene expression that occurs in all kinds of inflammatory contexts.

It is essential to elucidate the molecular mechanisms that promote cell survival or induce death in response to inflammatory stimuli for the better understanding of the pathogenesis of inflammatory diseases and to be able to identify novel therapeutic approaches. The AP-1 transcription factor complex which has a function in cell proliferation, cell transformation and differentiation during development as well as in adult tissues is also a key player in regulating inflammatory processes [4]. AP-1 is composed of homodimeric and heterodimeric complexes consisting of members of the Jun, Fos, musculoaponeurotic fibrosarcoma protein families and activating transcription factor [5]. It can be activated by cytokines, hormones, chemokines, multiple environmental stresses and growth factors. For instance, an activation of cascades which involves the stress- responsive mitogen-activated protein kinases such as Jun-n-terminal kinases, leads to an activation of AP-1[6]. The regulation of AP-1 complexes can occur at several levels including mrna translation, transcription and turn over including micro rnas (mirnas), protein stability or by interactions with other transcription factors [7]. Moreover, during development different ap-1 dimers are expressed in a cell- and stage- dependent manner.

Chronic inflammatory diseases are affecting a large number of populations. In addition, cancer and even metabolic diseases such as atherosclerosis or type 2diabetes are believed to have an

inflammatory component [8]. It is thought that many of these inflammatory diseases chemotactic or chemo attractant proteins and cytokines are released at the side of injury or infection, which then attracts innate and adaptive immune cells. The cytokine milieu together with the immune cells activates a cascade of events, called the inflammatory process. Many cytokine genes are regulated cooperatively by a transcription factor complex consisting of AP-1. IL-2, IL-3, granulocyte–macrophage colonystimulating factor, IL-4, IL-5, IL-13, IFNɣ, TNFα, CD40L, fasl, CD5, Igκ, CD25 and the chemokines IL-8 and MIP1α. Importantly, for the majority of these genes, the induction with AP-1 appears essential. The innate immune system utilizes cellular components such as macrophages or dendritic cells and humoral components of the complement system to respond to infectious agents. The activation of Toll-like receptors is an essential starting point for the activation of innate immunity. Once Activated, Toll-like receptors lead among other events to the differentiation of macrophages and to the production of several cytokines such as TNFα, IL-1, IL-6 or IL-12. The signaling of Toll-like receptors leading to cytokine production integrated by adapter molecules such as TRAF6 and myd88 that eventually activate AP-1 and NF-kB [9]. Allergic asthma, psoriasis and RA are thought to be inflammatory diseases mediated by activated T cells. AP-1 has been shown to be involved in the differentiation of native T cells into T helper 1 cells and T helper 2 cells, which is an indicator of the T cell-dependent immune response. During differentiation, Jun B positively regulates IL-4 expression and accumulates in T helper 2 cells [10]. In agreement, loss of Jun B in polarized T helper 2 cells in vitro which is followed by deregulated expression of T-helper-2-specific cytokines and by expression of T-bet and IFNɣ are known as key regulators of T helper 1 cells [11]. By the molecular mechanism Jun and Jun B regulate T helper 2 cytokine expression has been identified. After targeting for degradation by the E3-ligase Itch in a jnk dependent pathway, the turnover of Jun and Jun B is regulated by ubiquitin-dependent proteolysis [12]. Due to reduced expression of IL-4, CD25 and CD69 ectopic over expression of Jun D suppresses T cell proliferation and activation [13], together, these data incriminate Jun proteins as a vital player in T cell-mediated diseases that are characterized by an imbalanced ratio of T helper 1 effector cells and T helper 2 effector cells. Glucocorticoids are very effectual in controlling inflammation and are used for the treatment of autoimmune diseases such as RA. Expression of several cytokines such as IL-1, IL-2 or IFNɣ is activated by AP-1 and other transcription factors, but is oppressed by the glucocorticoid receptor (GR). The data suggest that the GR prevents the interaction between DNA-bound AP-1 complexes and transcriptional coactivators. Irrespective of the exact mechanism, the ability of the GR to repress the proinflammatory transcription factors AP-1 seems the most important function of the GR.

AP-1 factor plays very important role in many inflammatory diseases. It has become the therapeutic target for a number of inflammatory diseases. Therefore, various strategies can be employed to inhibit the AP-1 factor. Developing small molecule inhibitor could be an attractive strategy. In this connection, one of the studies developed a small molecule called T-5224, which is under clinical phase-II. There is constant demand to develop the small molecule inhibitor for better management of inflammatory diseases. T-5224 is a novel AP-1 inhibitor, which has proven to prevent pannus formation, joint destruction and osteoclastogenesis in collagen-induced arthritis (CIA) in rats. It shows good result as a new drug for the treatment of arthritis and is currently in phase II human clinical trials in Japan. Glucocorticoids are the most widely prescribed and most effective anti-inflammatory medications that are presently available.

Therefore, the objective of the present study is to design a new small molecule inhibitor for AP-1 factor involved in inflammatory disease by *insilico* approach and followed by its validation studies. This study may help the scientific community and better management of inflammatory diseases.

OBJECTIVES

1. Structural characterization of Activator Protein-1.
2. Designing of small molecule inhibitors for AP-1 based on referral drug T-5224.
3. Identification of small molecule inhibitors of AP-1 based on phytochemicals.
4. Comprehensive screening of datasets for the identification of small molecule inhibitors for AP-1.
5. *In-silico* validation of shortlisted small molecule inhibitors.

Methodology

1) **Structure retrieval of AP-1 factor**

   The 3-D structure predidiction of AP-1 factor by using PDB database. Characterization and validating quality of AP-1 factor is based on stereochemistry of residue by residue geometry and overall structural geometry using structure analysis and verification server (SAVES). Finding active site of AP-1 by using CASTp database.

2) **Identification of Potent drug against inflammation by using *in-silico* technique**

   Different protein families of AP-1 factor like Jun and Fos will be tested towards binding efficiency with active site of AP-1, in order to inhibit its action. This will be carried out in suitable Docking Software and there by the results will be correlated with in-vitro studies.

3) Ligand preparation

Collection of existing ligand or drug molecules which are already used as potential drug molecules for the inhibition of AP-1 factor in inflammatory diseases and those drugs are retrived from PubChem compound database. The formulated ligand molecules will be modified on the bases of physical and chemical properties by using pharmacokinetic properties. By the end, the fragments will be identified on the basis of "Lipinski's Rule of Five" that represents a suitable strive for proficient lead compounds. Designing a new drug molecule by using MarvinSketch/ChemSketch software to add all chemical compositions and the final output will be saved in .mol2 format. The pharmacophore and pharmacokinetic properties of ligand molecule were studied, and Pharmacogenomic properties within biological properties were analyzed using Hyperchem 7.5 Professional. The resultant ligand molecules were used for molecular docking.

4) Molecular docking

Identifing and analysing the intraction between drugs and protein by using Auto Dock 4.2 software package. A set of Lamarckian genetic algorithm was used for molecular docking simulations. Each simulation was performed 10 times, yielding 10 docked conformations. The lowest energy conformations were regarded as the binding conformations between the ligands and the proteins. Further, the reverse validation processes ensure that the identified hits really fit the generated Pharmacophore models and active sites of both targets. All the parameters required for molecular docking and Pharmacophore mapping were fixed as used in regular process.

Timeline

First year

1. Literature survey and collection of data related to study.
2. Thorough reviewing of AP-1 protein families which are mainly expressing during inflammation and causing the inflammatory diseases.
3. Study about the small molecules structure which inhibits the AP-1 factor in inflammation.
4. Acquiring the knowledge of existing drug molecules for the inflammation.

Second year

5. Finding the interaction between the drug molecule and the proteins of AP-1 factor
6. Studying the binding properties of ligand and target.
7. Working on the structure of the drug molecule to get the exact binding site for predicting the small molecules for the inhibition of the AP-1 factor

Third year

8. Thorough molecular studies.
9. Insilico and Invitro validation studies.
10. Compilation of the collected data, preparation of manuscript and thesis writing.

Significance of study

This study is mainly focusing on the involvement of AP-1 factor in inflammation causing inflammatory diseases. The study also makes an effort to provide a new approach of finding a better drug molecule for the treatment of inflammatory diseases.

Expected outcome

- The insight of understanding the role of AP-1 in relapses of inflammatory diseases.
- *Insilico* approach on binding efficiency of certain drug molecules with protein families of AP-1 transcription factor and therefore providing a new approach towards drug design for treatment of inflammatory diseases.
- Opens up a new window to design a drugs for the treatment of inflammation

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Signature of candidate **Signature of guide**